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Eijkenboom, M; Gerlach, [No Value]; Barker, A; Luiten, P; Van der Staay, FJ; Gerlach, I.

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CHRONIC COGNITIVE EFFECTS OF BILATERAL SUBDURAL HAEMATOMAS IN THE RAT

M. EIJKENBOOM,[a]* I. GERLACH,[a] A. BARKER,[b] P. LUITEN[c] AND F. J. VAN DER STAAY[a]

[a]*Central Nervous System Research, Bayer Healthcare, Aprather Weg 18a, 42906 Wuppertal, Germany*

[b]*Amaxa GmbH, Cologne, Germany*

[c]*Department of Molecular Neurobiology, University of Groningen, Groningen, The Netherlands*

Abstract—Humans suffering from subdural haematomas often show long-term cognitive dysfunctions. For identifying putative, recovery-enhancing therapeutics, animal models need to be developed in which recovery of function can be measured. For investigating whether and which type of recovery, i.e. spontaneous or training-induced recovery, or continuous partial retardation, is present in the rat model for bilateral subdural haematomas, spatial navigation abilities were assessed in the Morris water escape task in independent groups of rats at 1, 2, 4, 8, or 18 weeks after surgery. Complete spontaneous recovery seemed to occur at 8 weeks after injury. However, at 18 weeks after injury, the subdural haematoma caused a renewed deterioration of water maze performance, which was of a lesser degree than the impairments observed immediately after injury. This second phase performance deterioration was accompanied by an increase in generalised astrocyte reactivity. The rat subdural haematoma model provides an interesting tool for investigating spontaneous recovery processes of spatial navigation (8 weeks after injury), but also for progressive brain dysfunctions, considering the second phase of behavioural impairments seen at 18 weeks after injury.

Key words: animal model, cross-sectional design, Morris water escape task, recovery of function, spatial learning.

Patients with spontaneously occurring or impact-induced subdural haematomas (SDH) might suffer from long-term cognitive deficits (Levin et al., 1988; Whyte et al., 1994). Rehabilitation programmes, which support motor recovery successfully, are generally not suitable for restoring functions in brain-injured patients with persistent cognitive dysfunctions (Jørgensen et al., 1995; Gresham et al., 1997). For developing effective therapies, animal models are required in which recovery of cognitive function can be investigated. Here, the chronic effects of bilateral SDH on spatial learning and memory in the rat are evaluated and discussed as a tool for investigating cognitive recovery and identifying novel therapeutics.

The rat model for SDH was originally developed by Miller et al. (1990). In this model, autologous blood is injected underneath the dura, forming a haematoma. As in SDH patients (Graham et al., 1978, 1987; Adams et al., 1983), cortical lesions and damage to the hippocampal formation have been observed in SDH rats (Bullock et al., 1991; Kuroda and Bullock, 1992; Doppenberg et al., 1999). Damage to these brain structures has been associated with cognitive decline in patients (Graham et al., 1978; Adams et al., 1983).

Cognitive, as well as motor deficits have been reported for the rat SDH model. SDH placed bilaterally above the sensorimotor cortex in the rat induced attention deficits, as measured in a visual discrimination task (Klapdor et al., 1997), and spatial acquisition impairments, as measured in the allocentric version of the Morris water escape task (Eijkenboom et al., 1999, 2000b). Although SDH restricted to one hemisphere caused stable reflexive (at least 5 weeks after injury) and skilled sensorimotor deficits (at least 3 weeks after injury; Eijkenboom et al., 2000b), unilateral SDH did not affect Morris water maze performance (Eijkenboom et al., 1999).

In previous studies (Eijkenboom et al., 1999, 2000b), we observed that the spatial learning ability of bilateral SDH rats was not abolished, although significantly impaired when testing started at 1 week after surgery. For instance, the SDH rats were capable of acquiring the Morris task to control level, but they needed 10, instead of four (control rats) training sessions for reaching the level of maximum performance. Moreover, after the SDH rats had mastered navigating to one particular platform position, the platform was put in a new location and a reversal training was started. In the reversal training the SDH rats were only slightly impaired, i.e. SDH rats reached maximum performance during the fourth session, whereas control rats managed this during the second session. Because relearning and general task experience are confounding factors in the Morris water escape task with repeated testing (Pitsikas et al., 1991; Gyger et al., 1992; van der Staay and Blokland, 1996), we could not differentiate whether the observed performance improvement during training reflected continuous retarded spatial learning (slow learning), or spontaneous and/or training-induced recovery. In the present study, for investigating whether spontaneous cognitive recovery occurs in SDH rats, we chose a cross-sectional design, i.e. independent groups of rats started the Morris task at five different post-surgery intervals (1, 2, 4, 8, and 18 weeks). In parallel to behavioural testing,

*Corresponding author. Tel: +49-202-365-106; fax: +49-202-36969-5106.
E-mail address: eijkenboom_cns@yahoo.de (M. Eijkenboom).

Abbreviations: ANOVA, analysis of variance; GFAP, glial fibrillary acidic protein; PBS, phosphate-buffered saline; SDH, subdural haematoma groups; SH, sham-operated groups.

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reactivity of astrocytes (as an indicator for secondary responses to injury) and lesion location were followed with the aid of histological methods.

EXPERIMENTAL PROCEDURES

Animals

One hundred fifteen male Wistar rats (weighing 220–250 g) were obtained from Harlan-Winkelmann (Borchen, Germany) in batches of 23 rats each. One batch of rats was used per post-surgery interval (1, 2, 4, 8, and 18 weeks). Animals were housed in pairs in standard Makrolon type III cages and allowed to habituate to the animal room for 1 week before surgery. The housing conditions were: temperature 21.5±1.5 °C, humidity 50%, lights on from 7.00 a.m. until 7.00 p.m. with a radio playing quietly. Food (Ssniff rat food pellets) and water were available *ad libitum* throughout the study.

On the day of surgery, of the 23 rats in a batch, 20 rats were randomly assigned to behavioural testing. Three rats were used for histological verification of bilateral SDH lesions. The rats assigned to behavioural testing were then randomly divided into sham-operated (SH) or SDH groups. The following groups (n=8–11) for behavioural testing were formed: SH1, SDH1, SH2, SDH2, SH4, SDH4, SH8, SDH8, SH18, and SDH18. The numbers assigned to the abbreviations correspond to the different surgery-test intervals in weeks. Before surgery, rats were routinely tested for impairments in grasping reflexes of the hind paws and in forelimb flexion (see below). None of the rats were impaired in these two sensorimotor functions before surgery.

One week before behavioural testing started, all rats, except for the SDH1 and SH1 groups, were transferred to the experimental room where they were housed for the entire testing period. The rats assigned to the 1-week surgery-test interval were transferred to the experimental room immediately after recovery from anaesthesia. The housing conditions in the experimental room were identical to those in the animal facility. The animals remained undisturbed during the surgery-test intervals except for routine animal care handling twice per week.

Animal care was according to protocols and guidelines approved by the European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize the number of animals used and their suffering.

Surgery

SDH was induced according to the surgical procedure of Miller et al. (1990) with some modifications (Eijkenboom et al., 1999). In short, during deep anaesthesia (mixture of nitrous oxide-oxygen and 1.5–3% isoflurane; Forene; Abbott GmbH, Wiesbaden, Germany), burr holes (diameter 3 mm) were drilled into the skull above the sensorimotor cortex (coordinates: 1 mm posterior and ±2.8 mm lateral from bregma). Specially designed plastic cannula (inner diameter: 0.4 mm; outer diameter: 0.8 mm) were lowered into the subdural space and fixed with tissue glue (Histoacryl; Braun, Melsungen, Germany). Subsequently, autologous non-heparinised blood was collected by puncture of the tail vein and was immediately injected via the prefixed cannulae into the subdural space (total volume: 100 μl per side, each injection lasted 4 min). Sham surgery involved anaesthesia and the midline scalp incision. Finally, the skin was sutured. After recovery from anaesthesia the rats were returned to their home cages.

All rats recovered well after surgery, with the exception of one SH animal assigned to the 8 week surgery-test interval which died from unknown causes. No differences in gross behaviour were observed between the SH and the SDH groups.

Verification of surgery (Gerlach et al., 1998)

A successful placement of the haematoma was verified by testing the grasping reflexes of the hind paws and the forelimb flexion in all rats, 1 or 2 days after surgery.

Forelimb flexion. The rats were lifted by their tails and lowered toward a table until the forepaws nearly reached the surface. Special care was taken that neither the vibrissae nor other parts of the body contacted the table. In this position, unlesioned rats stretch out both forelimbs, which was scored as 1. Impaired rats keep the contralateral forelimb flexed, which was scored as zero (modified after Marshall, 1982).

Grasping reflex of the hind paws. Rats were held loosely in one hand, with thumb- and index-finger around their chest, immediately under the forelegs. Then, the experimenter gently touched the paw between the first and second digits with a thin round brush (diameter 5 mm). A normal, intact rat grasps the brush immediately. This behaviour was scored as 1. Failure or delayed grasping was scored as being impaired and was awarded the score zero. Both hind paws were tested (modified after van der Staay et al., 1996).

All testing was done blind, that is, the experimenter was not informed of the kind of surgery. Sensorimotor impairments (forelimb flexion and grasping reflex) in SDH rats were seen as an indication of a successful placement of the SDH over the sensorimotor cortex (Gerlach et al., 1998). Absence of these dysfunctions in SDH-animals served as exclusion criterion; in SH-rats the presence of these dysfunctions served as exclusion criterion. No animals were excluded based on the sensorimotor tests.

Spatial navigation testing

The spatial navigation testing section incorporated a nonspatial swimming training in an aquarium, followed by five training sessions and five overtraining sessions in the standard Morris water escape task. After both series of training and overtraining a probe trial was given.

Aquarium training. Three days before the Morris water maze procedure started, all rats were subjected to 5 min of nonspatial swimming training in a glass aquarium (98.3×49.3×38.1 cm) filled to a height of about 35 cm with water at room temperature. This training was included for avoiding differences between groups in water maze performance due to initial swimming problems. All rats were able to perform in the aquarium training.

Morris water escape task. The ability to locate a submerged platform by using allocentric strategies was tested in a Morris water escape task. The Morris maze consisted of a circular tub, painted grey. The tub had a slightly sloping wall (material: polyethylene; inner dimensions: diameter at top 153 cm, diameter at bottom 143 cm, depth 63 cm) and was filled with clear tap water at a temperature of approximately 22 °C. A grey polyethylene cylinder (diameter 10.8 cm), submerged 1.5 cm below the surface of the water, served as escape platform.

A video camera, mounted in the centre above the circular pool, provided a picture of the pool on a TV monitor. The position of the rat was sampled five times per second. The movements of the rats were registered using the automatic video tracking system EthoVision (Noldus Information Technology, Wageningen, The Netherlands).

During each of the five daily acquisition and overtraining sessions, the animals performed four trials, which were given in close succession. A trial was started by placing the rat into the pool, facing the wall of the tank. Each of the four starting positions (arbitrarily assigned north, east, south and west) was used once in a series of four trials; their order was randomised. The escape

platform was always in the middle of quadrant West. A trial was terminated as soon as the rat climbed onto the escape platform or when 90 s had elapsed, whichever event occurred first. Each rat was allowed to stay on the platform for 30 s. Then it was removed from the platform and the next trial was started. Rats that did not find the platform within 90 s were put on the platform and were allowed to stay there for 30 s. The animals were subjected to 1 session per day. The overtraining sessions started 3 days after the last training session.

After the fourth trial of the fifth acquisition session and the fourth trial of the fifth overtraining session, the retention of the platform position was tested in a probe trial. During the two probe trials the platform was removed, and the time a rat spent in the four quadrants was measured for 30 s. The area around the previous platform position was defined as the annulus region (diameter 40 cm). The time animals spent in this region and the crossings of this region were registered. All rats started from the same position, opposite to the quadrant where the escape platform had been positioned during acquisition.

After completion of the last trial of each daily session the rats were dried and returned to their home cages. There the animals were kept warm for 5–10 min under an infrared lamp (Original Hanau Solilux, Hanau, Germany; 150 W) fixed about 60 cm above the floor of the cage.

Histological verification of the lesion

Three rats per surgery-test interval were assigned to parallel histological verification, i.e. on the third day of Morris water escape testing of the rats assigned to behavioural testing, these three rats were killed. The animals were deeply anaesthetized with barbiturate (Narcoren) and transcardially perfused with phosphate-buffered saline (PBS) for 1 min followed by a fixative (1% glutaraldehyde and 3% paraformaldehyde in PBS) for 10 min. After removal, the brain was postfixed for 1 h in the same fixative. The brains were trimmed in the frontolateral plane at six levels and embedded in paraffin. Paraffin sections were stained with haemalum and eosin or Luxol-Fast Blue/Cresyl Violet. Moreover, in a second series of sections, glial fibrillary acidic protein (GFAP; BioGenex, San Ramon, CA, USA, Clone GA-5; 1:200; detected with Vectastain ABC Kit) labelling was done in deparaffinized sections which were hydrated and pre-incubated for 30 min with 3% H_2O_2 to block endogenous peroxidase activity. Calcium deposits were stained according to the method of Kossa (Romeis, 1989). The lesion sites and sizes were determined by microscopic examination of haemalum and eosin-stained sections. The lesioned structures were assessed according to Swanson's (1998) rat brain atlas.

Statistical analysis

Acquisition and overtraining in the Morris water escape task. The following four parameters for acquisition and overtraining in the water escape task were analysed statistically: the escape latency (s); the distance travelled (cm); the swimming speed (path length divided by escape latency; cm s^{-1}); and the distance to platform (average distance between a rat and the platform; cm). All data were assessed with a Lesion (with the levels SH and SDH) by Surgery-test interval (with the levels 1, 2, 4, 8, and 18 weeks post-surgery) by Session (with the levels session 1–5 for the acquisition series, and 6–10 for the overtraining series) analysis of variance (ANOVA) with repeated measures over sessions (SAS GLM-procedure; Freund and Littell, 1985; Cotton, 1998). Moreover, the mean performance level, i.e. average across the five sessions, for the four measures at a particular surgery-test interval was expressed as per-cent of the mean performance level of the SH group at that particular interval, applying the formula: $P_{i,n}=(X_{i,n} - \overline{X}_{SH,n})/\overline{X}_{SH,n}*100$, where $P_{i,n}$ is the percentage deviation of a particular animal i at Surgery-Test interval n, $X_{i,n}$ is the observation of a particular animal i at interval n, and $\overline{X}_{SH,n}$ is the mean of a particular measure of the sham-group at interval n. Whether the mean performance on a particular test series differed from zero at the independent surgery-test intervals was determined with Student's t-test.

Probe trial. Effects of the lesion and the different post-surgery intervals on the bias for particular quadrants during the probe trials were assessed with a Quadrants (south, west, north, and east being considered as levels of the repeated measures factor Quadrants) by Lesion by Surgery-test interval ANOVA, with repeated measures on the first factor. The effects of the lesion on the time spent, and the distance travelled in the training quadrant were analysed by ANOVA per surgery-test interval whenever statistically reliable interactions of Quadrants with Surgery-test interval or Lesions were found. In addition, the effects of lesions and surgery-test intervals on the time spent in the annulus were analysed by a Lesion by Surgery-test interval ANOVA.

RESULTS

Histological verification

The SDH induced lesions comparable to those reported in previous studies, including ventricle dilations and subsequent compression damage (Eijkenboom et al., 1999, 2000b). A typical haematoma is shown in Fig. 1. However, in this study we observed additional pathological changes, of which two are highlighted here.

First, from 8 weeks after injury onwards, calcium deposits developed in the thalamic nuclei (see Fig. 1, panel C). At shorter surgery-test intervals these brain regions were strongly labelled with GFAP, indicating strong astrocyte reactivity. Second, brain areas remote from the haematoma (e.g. hippocampus, thalamus, and optical tract) were labelled for GFAP at 1, 2, and 4 weeks after injury. At 8 weeks after injury the generalised GFAP labelling was absent. However, an increase in astrocyte reactivity higher and more generalised than in the fourth week after injury was observed at 18 weeks after injury, i.e. now also the piriform cortex, entorhinal cortex and ventricle surroundings were labelled. This pattern in time of generalised GFAP labelling is visualised for the hippocampus in Fig. 2. GFAP labelling surrounding the haematoma was high at every surgery-test interval.

Spatial learning: acquisition and overtraining

The results concerning the dynamics in the performance of the groups with increasing length of the surgery-test interval are discussed in detail in the following paragraphs. In Tables 1 and 2, respectively, the outcomes are described for the acquisition sessions and the probe trials for each separate post-surgery interval.

We found that the latency to escape onto the platform as a measure for spatial acquisition was biased by differences in swimming speed between groups and surgery-test intervals. The distance travelled before escaping was, therefore, a less biased and more valid measure for spatial orientation performance.

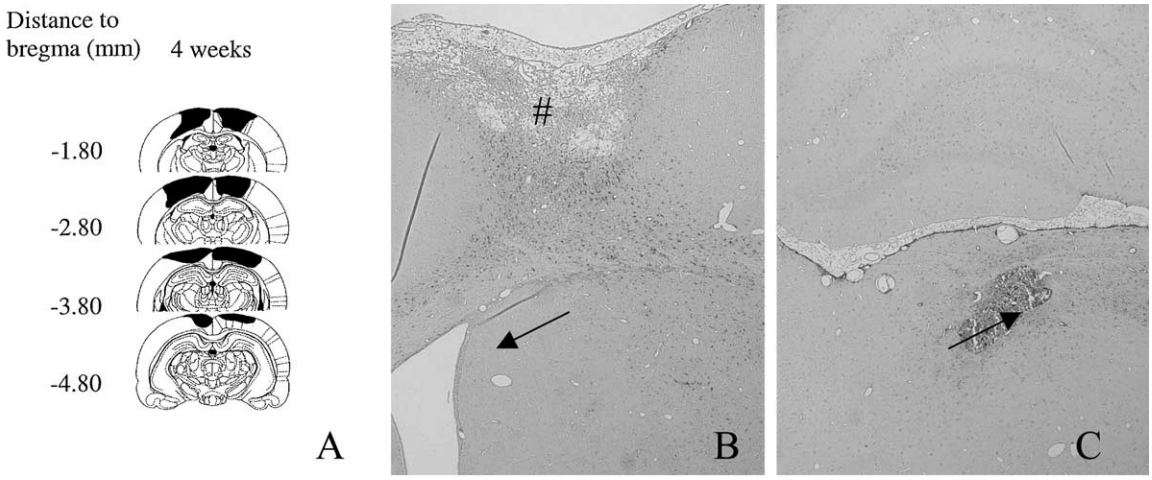

Fig. 1. Panel A depicts a typical example of lesion location at 4 weeks after the induction of a bilateral SDH (black areas; drawings of the brain sections were reproduced from Paxinos and Watson (1998) with permission). Distance to bregma is depicted on the left side of the figure. In panel B, a haematoma (#) in the sensorimotor cortex of the right hemisphere is shown with the accompanying ventricle dilation at 2 weeks after surgery (arrow, at approximately 0.20 mm to bregma). An example of a calcium deposit in a thalamic nucleus at 8 weeks after bilateral SDH surgery can be seen in panel C (arrow, at approximately −3.80 mm to bregma).The pictures were taken from GFAP-stained sections.

Distance travelled (Fig. 3, first row)

Acquisition. Averaged over the standard acquisition sessions, SDH rats used more elaborate paths to reach the escape platform than the SH rats did (Lesion: $F_{1,88}=39.24$, $P<0.001$). The effect of the lesion diminished with increasing length of the surgery-test interval ($F_{4,88}=2.96$, $P<0.05$), although the post-surgery interval did not influence the distance swum averaged over all sessions directly ($F_{4,88}=1.25$, n.s.). The distance travelled to reach the platform decreased across sessions ($F_{4,352}=161.66$, $P<0.001$), demonstrating spatial learning. The decrease in distance swum, i.e. the rate of learning, was slower after SDH surgery than after SH surgery (Session by Lesion interaction: $F_{4,352}=8.32$, $P<0.001$), without any influence of the time after surgery (Session by Surgery-test interval interaction: $F_{16,352}=1.45$, n.s.; Session by Lesion by Surgery-test interval interaction: $F_{16,352}=1.00$, n.s.).

The percentages of deviation (see Fig. 4, upper left panel) showed that only at the 8 week surgery-test intervals, the mean path length of the SDH group was not different from the mean SH performance (SDH8: $t_{10}=-0.36$, n.s.). At all other intervals the SDH rats performed worse than the SH animals, i.e. the deviation scores were different from zero (SDH1, 2, 4, 18: $t_9>3.40$, $P<0.01$).

Overtraining. The distance swum averaged over all overtraining sessions was longer for the SDH groups than for the SH groups (Lesion: $F_{1,88}=17.94$, $P<0.001$). The length of the surgery-test interval did not affect the session

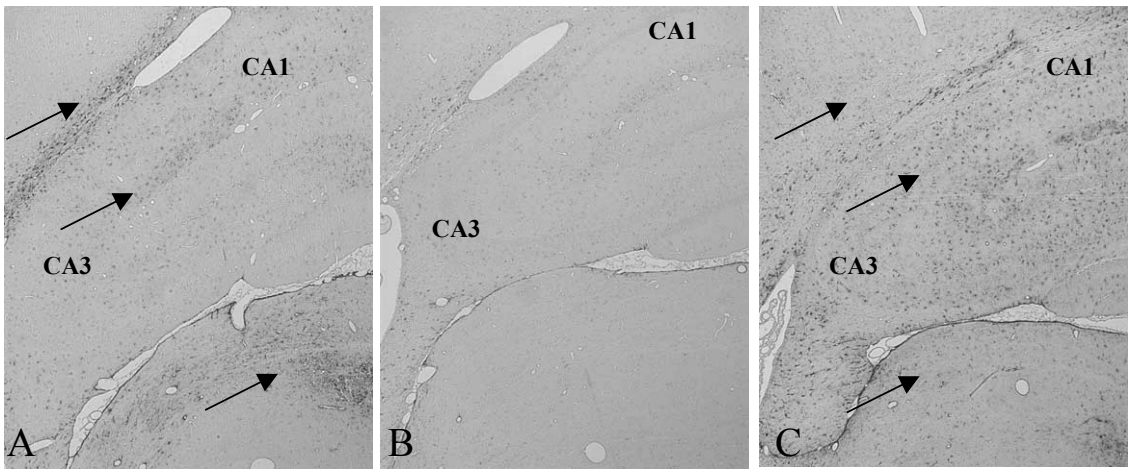

Fig. 2. Post-injury interval dependent patterns in generalised GFAP labelling are depicted for the hippocampal area (approximately −2.80 mm to bregma) at different post-injury intervals for rats with bilateral SDH. At 4 weeks (panel A) a mild generalised GFAP labelling (arrows) was seen, which was absent at 8 weeks after injury (panel B), whereas at 18 weeks after the induction of SDH the GFAP labelling was stronger and more generalised than at the 4 week interval (panel C, arrows).

Table 1. The chronic effects of bilateral SDH were compared with the effects of SH during a standard allocentric Morris water maze acquisition (sessions 1 to 5) and overtraining (sessions 6 to 10), starting at five independent surgery-test intervals[a]

Post-surgery interval	1 Week	2 Weeks	4 Weeks	8 Weeks	18 Weeks
Acquisition					
Between	$F_{1,18}=16.1^{\#}$	$F_{1,18}=13.1^{\#}$	$F_{1,17}=10.5^{*}$	$F_{1,17}=0.3$, n.s.	$F_{1,18}=4.9^{*}$
Within	$F_{4,72}=2.0$, n.s.	$F_{4,72}=2.5^{\circ}$	$F_{4,68}=2.2^{\circ}$	$F_{4,68}=0.7$, n.s.	$F_{4,72}=7.8^{\#}$
Overtraining					
Between	$F_{1,18}=5.3^{*}$	$F_{1,18}=3.7^{\circ}$	$F_{1,17}=6.5^{*}$	$F_{1,17}=0.4$, n.s.	$F_{1,18}=3.7^{\circ}$
Within	$F_{4,72}=2.8^{*}$	$F_{4,72}=3.1^{*}$	$F_{4,68}=4.1^{\#}$	$F_{4,68}=0.1$, n.s.	$F_{4,72}=0.7$, n.s.

[a] The length of the post-lesion intervals at which the independent experiments were started are 1 week, 2, 4, 8, and 18 weeks. Details of the statistical analyses (Between- and Within-subjects effects, or rather the general performance averaged across all measuring points and the changes over time or the shape of the learning curves, respectively) for each surgery test interval are described for the distance swum before escaping onto a submerged platform measured at five post-lesion intervals. In Figs. 3 and 4 these data are depicted as line-graphs. A description of the repeated measures analyses on the scores per individual surgery-test interval can be found in Eijkenboom et al. (1999).
$^{\circ}$ $0.10>P>0.05$; * $P<0.05$; $^{\#}$ $P<0.01$.

average of the distance travelled across the overtraining sessions (Surgery-test interval: $F_{4,88}=0.49$, n.s.; Lesion by Surgery-test interval interaction: $F_{4,88}=1.60$, n.s.). In the course of the overtraining, all rats shortened the path length before escaping onto the platform (Sessions: $F_{4,352}=26.16$, $P<0.001$). The decrease in the distance swum was faster in the SDH group than it was in the SH animals (Session by Lesion interaction: $F_{4,352}=8.55$, $P<0.001$). Note, that the latter groups had already reached the learning asymptote during acquisition. The length of the surgery-test interval had no influence on the dynamics of the development of performance over sessions (Session by Surgery-test interval interaction: $F_{16,352}=0.36$, n.s.; Session by Lesion by Surgery-test interval interaction: $F_{16,352}=0.79$, n.s.).

A statistically significant difference between the mean SDH overtraining performance and the mean SH overtraining performance was only observed at 4 and 18 weeks (see Fig. 4, upper right panel; SDH4,18: $t_9>2.39$, $P<0.05$; SDH1, 2, 8: $t_{9-10}<2.20$, n.s.).

Distance to platform (Fig. 3, second row)

Acquisition. Averaged over the acquisition sessions, the distance to the platform was affected by the lesions and not by the time after surgery (Lesion: $F_{1,88}=4.58$, $P<0.05$; Surgery-test interval: $F_{4,88}=1.48$, n.s.; Lesion by Surgery-test interval interaction: $F_{4,88}=1.92$, n.s.). During the training, the distance to the platform diminished (Session: $F_{4,352}=26.29$, $P<0.001$) differently for the surgery-test intervals (Session by Surgery-test interval interaction: $F_{16,352}=5.98$, $P<0.001$). The effect of the time after surgery was more pronounced in the SDH groups than in the SH groups (Session by Lesion interaction: $F_{4,352}=1.87$, n.s.; Session by Lesion by Surgery-test interval interaction: $F_{16,352}=3.87$, $P<0.001$).

The *t*-test on percentages of deviation from SH means demonstrated that only at 4 and 18 weeks after surgery the SDH rats swam at a greater distance from the escape platform than the SH rats did (see Fig. 4, centre left panel; SDH2,18: $t_9>2.64$, $P<0.05$; SDH1,4,8: $t_{9-10}<-1.92$, n.s.).

Overtraining. The averaged distance to the escape platform during overtraining sessions was different for the groups, but not for the post-surgery interval by itself (Lesion: $F_{1,88}=16.46$, $P<0.001$; Surgery-test interval: $F_{4,88}=0.33$, n.s.), i.e. the SDH rats swam at a greater distance from the escape platform than the SH animals did. The lesion, however, differently affected the distance to platform across surgery-test intervals (Lesion by Sur-

Table 2. The chronic effects of lesions due to bilateral SDH were compared with the performance of SH groups of rats in the allocentric version of the Morris water escape task[a]

Post-surgery interval	1 Week	2 Weeks	4 Weeks	8 Weeks	18 Weeks
Probe I					
Time in quadrants: Quadrant×Lesion interaction	$F_{3,54}=9.3^{\#}$	$F_{3,54}=2.1$, n.s.	$F_{3,51}=9.7^{\#}$	$F_{3,51}=0.7$, n.s.	$F_{3,54}=1.6$, n.s.
Time in annulus: Lesion effect	$F_{1,18}=11.9^{\#}$	$F_{1,18}=5.8^{*}$	$F_{1,17}=15.3^{\#}$	$F_{1,17}=0.2$, n.s.	$F_{1,18}=0.0$, n.s.
Probe II					
Time in quadrants: Quadrant×Lesion interaction	$F_{3,54}=2.2$, n.s.	$F_{3,54}=4.0^{*}$	$F_{3,51}=0.3$, n.s.	$F_{3,51}=3.4^{*}$	$F_{3,54}=0.7$, n.s.
Time in annulus: Lesion effect	$F_{1,18}=1.0$, n.s.	$F_{1,18}=3.0$, n.s.	$F_{1,17}=0.7$, n.s.	$F_{1,17}=6.3^{*}$	$F_{1,18}=3.5^{\circ}$

[a] The bias for the previous platform position was assessed during two probe trials in each of five independent surgery-test intervals. The length of the post-lesion intervals at which the independent groups were started are 1 week, 2, 4, 8, and 18 weeks. Statistical details are described per surgery-test interval for the time(s) spent in the quadrant in which the escape platform had previously been positioned and the time (s) spent in the annulus region. In Fig. 5 these data are depicted in bar-diagrams.
$^{\circ}$ $0.10>P>0.05$; * $P<0.05$; $^{\#}$ $P<0.01$.

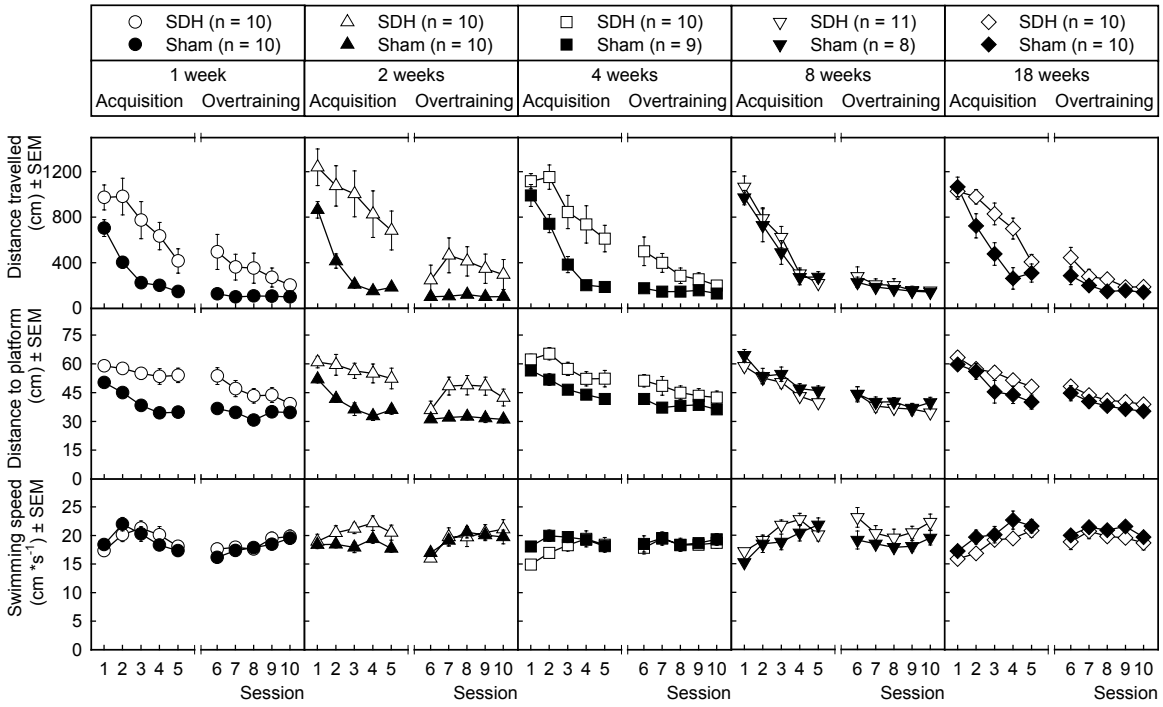

Fig. 3. Acquisition of the position of a submerged platform in the Morris water maze in sham operated rats (SH) and rats with bilateral SDH. Testing started 1, 2, 4, 8, or 18 weeks after surgery for independent groups. Group numbers correspond to the respective post-lesion intervals. The training is divided into two periods: acquisition (sessions 1–5) and overtraining (sessions 6–10). Session means and S.E.M. of the distance travelled (first row), the distance to platform (second row), and the swimming speed (third row) are depicted.

gery-test interval interaction: $F_{4,88}=3.00$, $P<0.05$). Over sessions, the rats learned to swim less far from the platform (Session: $F_{4,352}=31.89$, $P<0.001$). This decrease was dependent on the type of surgery, but was not affected by the post-surgery interval (Session by Lesion interaction: $F_{4,352}=3.27$, $P<0.05$; Session by Surgery-test interval interaction: $F_{16,352}=0.91$, n.s.; Session by Lesion by Surgery-test interval interaction: $F_{16,352}=1.03$, n.s.).

During the overtraining only at 1 and 2 weeks after surgery the SDH rats deviated from the SH rats in the mean performance (see Fig. 4, centre right panel; SDH1,2: $t>3.06$, $P<0.05$).

Swimming speed (Fig. 3, third row)

Acquisition. Averaged over all five acquisition sessions, the swimming speed was similar for the SDH and SH groups ($F_{1,88}=0.01$, n.s.), and for the different surgery-test intervals ($F_{4,88}=0.80$, n.s.). However, a combination of these two factors did affect the speed of swimming (Lesion by Surgery interaction: $F_{4,88}=2.62$, $P<0.05$). The speed of swimming increased over sessions (Sessions: $F_{4,352}=26.47$, $P<0.001$), differently for the groups (Session by Lesion interaction: $F_{4,352}=3.08$, $P<0.05$), and for the post-surgery intervals (Session by Surgery-test interval interaction: $F_{16,352}=4.42$, $P<0.001$). There was no interaction between the type of surgery and the post-surgery time ($F_{16,352}=1.51$, n.s.).

At 2 weeks after surgery, the mean swimming speed of the SDH rats during the acquisition training was higher than the SH swimming speed (see Fig. 4, lower left panel; SDH2: $t_9=2.52$, $P<0.05$), whereas at 18 weeks after surgery the SDH rats swam slower than the SH rats (SDH18: $t_9=-3.06$, $P<0.05$), as seen by *t*-test on deviation percentages (see Fig. 4). At no other surgery-test interval a difference between the two treatments was seen (SDH1, 4, 8: $t_{9-10}<-2.03$, n.s.).

Overtraining. Averaged over the overtraining sessions, there was no effect of type of surgery and/or length of the surgery-test interval on the speed of swimming (Lesion: $F_{1,88}=0.24$, n.s.; Surgery-test interval: $F_{4,88}=1.79$, n.s.; Lesion by Surgery-test interval interaction: $F_{4,88}=0.95$, n.s.). The swimming speed remained stable over sessions ($F_{4,352}=2.25$, n.s.).

The *t*-test on deviation percentages from SH overtraining mean showed that there was an equal rate of swimming for the two treatments (see Fig. 4, lower right panel; all $t_{9-10}<-1.83$, n.s.).

Spatial learning: time in quadrants during probe trials (Fig. 5; first row of graphs)

First probe trial. The time the rats spent in the four quadrants was different (Quadrant: $F_{3,264}=273.88$, $P<0.001$). The preference for the quadrants was affected by SDH ($F_{3,264}=12.04$, $P<0.001$), by the post-surgery interval ($F_{12,264}=2.38$, $P<0.01$), and by an interaction between these two factors ($F_{12,264}=3.19$, $P<0.001$). The time spent in the training quadrant was affected by the lesion ($F_{1,88}=18.21$, $P<0.001$), i.e. the SDH rats spent less time in the quadrant in which the platform had been positioned during training than

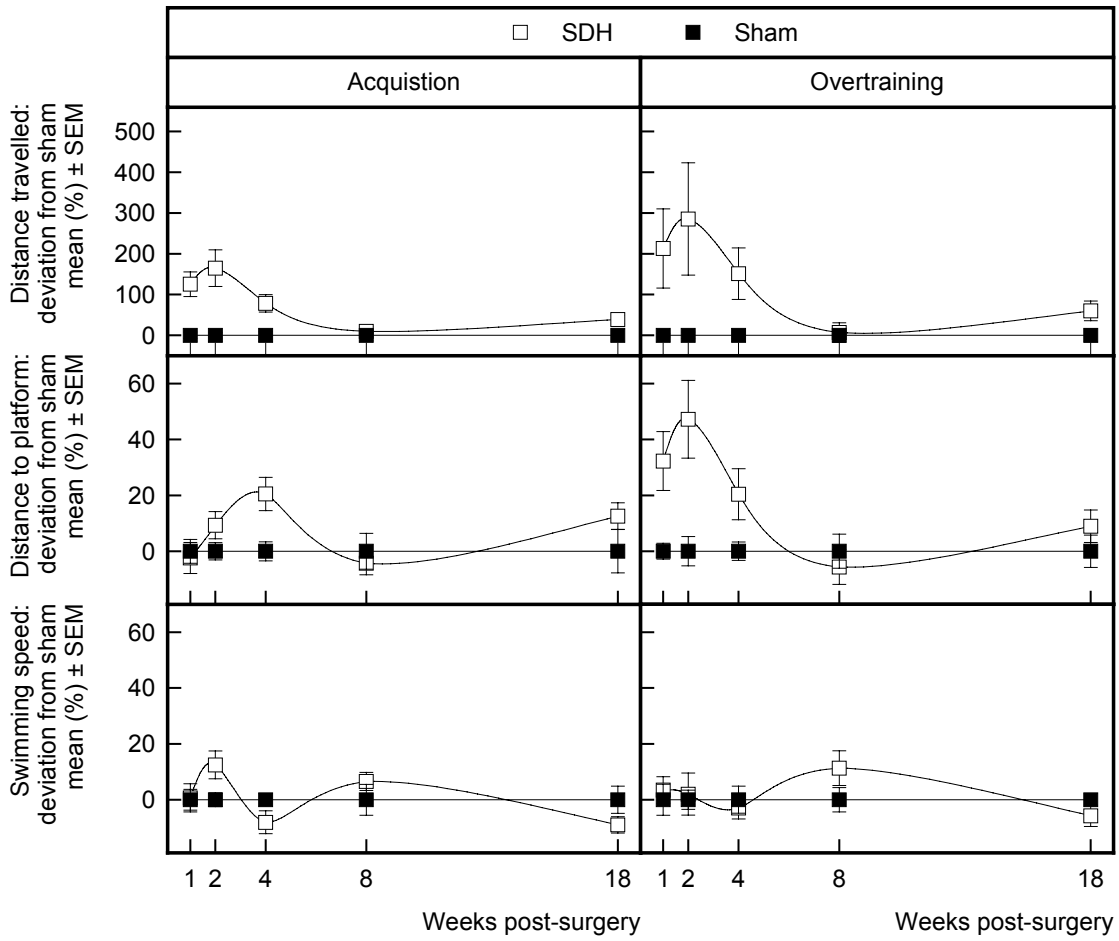

Fig. 4. Performance of independent groups of SH rats and rats with bilateral SDH in the Morris water escape task 1, 2, 4, 8, or 18 weeks after surgery. The percentage of deviation from the sham mean acquisition and overtraining performance, and the S.E.M. are depicted for the distance travelled, the swimming speed, and the distance to platform for each surgery-test interval.

the SH rats did. The length of the post-surgery interval influenced the time spent in the training quadrant as well ($F_{4,88}=2.69$, $P<0.05$). Moreover, the lesion-effect diminished with increasing time after surgery (Lesion by Experiment interaction: $F_{4,88}=3.98$, $P<0.01$).

Second probe trial. The preference for the training quadrant and differences between lesion-groups remained (Quadrants: $F_{3,264}=374.70$, $P<0.001$; Quadrant by Lesion interaction: $F_{3,264}=6.64$, $P<0.001$), whereas the length of the surgery-test-interval did not affect this preference (Quadrant by Surgery-test interval interaction: $F_{12,264}=0.62$, n.s.; Quadrant by Lesion by Surgery-test interval interaction: $F_{12,264}=0.92$, n.s.). SDH rats spent less time in the training quadrant than the SH rats did ($F_{1,88}=10.45$, $P<0.005$), independent of the length of the period between surgery and behavioural testing (Surgery-test interval: $F_{4,88}=0.77$, n.s.; Lesion by Surgery-test interval interaction: $F_{4,88}=1.12$, n.s.).

Time in annulus (Fig. 5; Table 2). During the first probe trial, the SDH rats spent less time in the annulus region than the SH animals did (Lesion: $F_{1,88}=16.73$, $P<0.001$). The effect of the lesion was dependent on the length of the post-surgery interval (Lesion by Surgery-test interval interaction: $F_{4,88}=2.50$, $P<0.05$). However, the post-surgery interval did not directly affect the time in annulus (Surgery-test interval: $F_{4,88}=1.46$, n.s.). Similar results had been found during the second probe trial (Lesion: $F_{1,88}=11.43$, $P<0.005$; Surgery-test interval: $F_{4,88}=1.74$, n.s.; Lesion by Surgery-test interval interaction: $F_{4,88}=0.43$, n.s.).

DISCUSSION

Bilateral SDH, positioned above the sensorimotor cortical areas, caused spatial navigation deficits in the standard Morris water maze, i.e. the distance swum before localising the platform was larger in rats with bilateral SDH than in rats which had undergone SH surgery. The occurrence of a deficit and the character of the deficit were dependent on the duration of the interval between injury and start of the water maze task.

The long term effects of bilateral SDH on water maze performance

The effect of bilateral SDH on averaged acquisition (general mean) in the Morris water maze diminished with in-

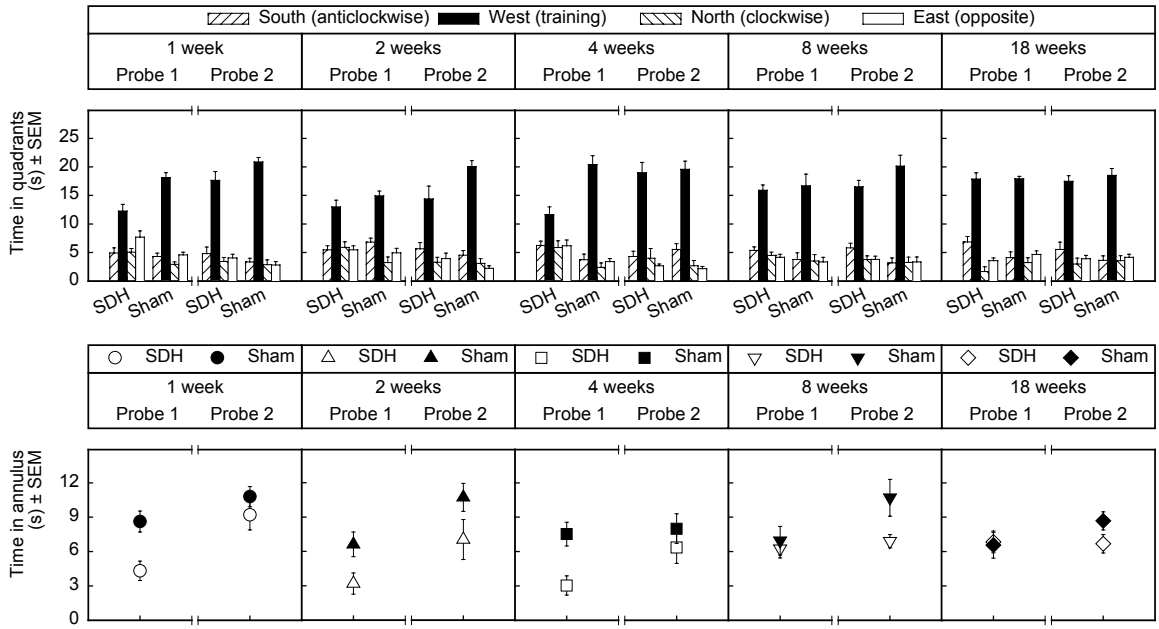

Fig. 5. The time spent in the four quadrants (upper row of panels) and in the annulus (lower row of panels) is depicted for rats with bilateral SDH and for SH animals during two probe trials in the allocentric version of the Morris water escape task, tested at five post-surgery intervals. The lengths of the post-lesion intervals at which the independent experiments were started are as follows: 1, 2, 4, 8, and 18 weeks. Means and S.E.M. are depicted.

creasing post-injury intervals, suggesting that partial spontaneous recovery occurred. However, looking at the group differences of the individual surgery-test intervals (see Table 1), the effect of post-injury time on haematoma-related water maze deficits was more dynamic. At each post-surgery time, except at 8 weeks after surgery, the SDH rats displayed a slower rate and a lower grade of acquisition than the respective SH rats did (see Table 1). At 8 weeks after surgery it appeared that the rats had completely recovered the capacity to spatially navigate. At 18 weeks after surgery, however, a renewed impairment in Morris water maze performance was seen. This biphasic recovery process was also observed for the percentage of deviation from the SH mean during the first training period. Fluctuations in recovery and deterioration of cognitive deficits have been reported for some patients after stroke-induced brain damage (Kotila et al., 1986).

Recovery of spatial navigation after bilateral SDH

One could argue that the SDH rats tested at 8 weeks after surgery were not impaired in the water maze task due to a failure of surgery. Although the positioning of the haematoma had been assessed with sensorimotor tests shortly after surgery, a proper comparison of the size of the lesions at the different surgery-test intervals would only have been possible with histological techniques. Our histological data, however, were derived from the brains of rats in parallel groups to the behavioural testing, which enabled us to investigate the character of the lesions at the time-point during which the other rats experienced the first period of acquisition. Still, the histological verification of the brains of rats at 8 weeks after surgery demonstrated the presence of a typical SDH lesion. Moreover, the replicability of the unexpected lack of deficits at 8 weeks after surgery was investigated in an independent study, with similar experimental conditions and again no behavioural deficit was found (see Fig. 6). In this replication study the performance of the SH rats was similar to the performance of the SH rats in the first 8 week-study (all *F*-values had associated probabilities >0.1).

We did not perform a second experiment at 18 weeks for replicating the renewed, mild disturbance in water maze performance seen at that surgery-test interval. However, the SDH rats at 18 weeks after surgery were very homogeneous in the degree of their deficit; thus, the degree of performance deficit appears to be real. Alternatively, the effect at 18 weeks might be the result of the random assignment. In that case, one must assume that the good performers were exclusively assigned to the SH group,

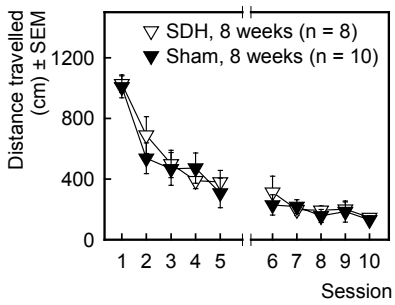

Fig. 6. Acquisition of the position of a submerged platform in the Morris water maze in sham operated rats and rats with bilateral SDH at 8 weeks after surgery. The results replicate the results at 8 weeks in Fig. 3. The training is divided into two periods: acquisition (sessions 1–5) and overtraining (sessions 6–10). Session means and S.E.M. of the distance travelled are depicted.

and all the poor performers to the SDH group, which cannot be excluded but is considered as highly unlikely.

The deficit at 18 weeks was not as pronounced as at 1, 2 and 4 weeks after surgery. During the overtraining sessions SDH18 rats performed as well as SH controls. The SDH1, SDH2, and SDH4 groups, by contrast, never reached the performance level of their respective SH-controls. This indicates that the performance deficits seen at 1, 2, and 4 weeks might be of a different nature than the deficit seen at 18 weeks after surgery. It would have been interesting to see how the impairment due to SDH would have evolved to a later time-point than tested here.

Comparing the performance during the first probe trial of each surgery-test interval, it became evident that SDH surgery resulted in smaller probe trial impairments with increasing post-injury times. At 8 and at 18 weeks after surgery, SDH rats and control rats showed a similar bias for the previous platform position (see also Table 2). The second probe trial yielded differences between the SDH and SH groups at some time-points. However, these differences are not likely to be related to spatial navigation deficits, as all rats had reached control performance levels at the end of the second training, immediately before the second probe trials were given. Probably, the motivation to search for the missing platform was decreased during the second probe trial and interfered with performance.

Histological verification

The results of the histological verification should be regarded with care, as they are deduced from a qualification, not a quantification, of only three subjects per surgery-test interval. Further, the brain material was from parallel animals that had not been subjected to the water maze procedures. Originally, we had designed to quantify changes in several biochemical markers in several brain areas of all the rats subjected to the water maze. However, during a laboratory move the homogenates were irretrievably lost. Thus, the results discussed here should only be seen as a guide for further studies using this deficit model.

Bilateral SDH surgery resulted in similar lesions as reported in previous studies (Eijkenboom et al., 1999, 2000b). Several interesting features were seen in the development of the lesion over the 18 weeks. First, ventricle enlargement and subsequent compression damage (e.g. in the hippocampus, striatum, thalamus, fimbria and corpus callosum) were observed at every post-surgery interval tested. Dilations of ventricles have been observed in humans (Lobato et al., 1988) and rats after head injury (Eijkenboom et al., 1999; Smith et al., 1997) and are probably related to brain swelling. The occurrence of brain swelling in SDH patients is highly associated with poor recovery (Lobato et al., 1988). Of course, a part of the ventricle dilation seen in the present study might also be related to a compensation of the brain for tissue loss.

Second, dynamics were seen in the strength and location of astrocyte reactivity (GFAP). At all surgery-test intervals astrocytes surrounded the haematoma, most likely related to clearing of debris. At 4 weeks after surgery, brain areas remote from the haematoma, such as the hippocampus, the thalamus and the optical tract were also strongly labelled for GFAP. These GFAP reactions have been associated with degeneration (Cook and Wisniewski, 1973; Bechmann and Nitsch, 1997) or denervation of the areas (Rose et al., 1976; Gage et al., 1988). After 8 weeks, the generalised astrocyte reactivity had disappeared and again only direct haematoma surroundings were labelled. At 18 weeks after surgery, a renewed high GFAP labelling could be observed surrounding the ventricles, in the hippocampus, the optical tract, the piriform cortex and the entorhinal cortex. Note that the fluctuations in GFAP labelling in areas remote from the haematoma coincided with the fluctuations seen in cognitive performance from 4 weeks onwards.

Third, calcium deposits in thalamic nuclei in SDH rats were found at 8 and 18 weeks. At shorter surgery-test intervals, reactive astrocytes had been seen in this location, instead of calcium deposits in the thalamic nuclei. Another trauma model, the rat fluid percussion injury model, has been reported to induce calcium deposits in the thalamus (Smith et al., 1997; Pierce et al., 1998). However in the gerbil repeated brief cerebral ischaemia model calcifications were found in more areas than only the thalamus (Araki et al., 1990). In humans, calcification of damaged cerebral tissue is known to occur after SDH (Erdem et al., 1994; Miyagi et al., 1995), but also after brain tumours, epilepsy, and cerebral infarction (Erdem et al., 1994). The accumulations of calcium in the thalamic nuclei after SDH in this study may reflect retrograde degeneration of projections between thalamus and cortex, when considering the extent of cortical and subcortical malfunctions induced by the infarcts.

The results obtained from the histological verification suggest that injury-induced dysfunctioning of the brain continues for at least 18 weeks after SDH, i.e. calcium accumulations, ventricle dilation and the occurrence of reactive astrocytes. Progressive degeneration and ventricular expansion have been reported after moderate controlled cortical impact (up to at least 2 months: Matthews et al., 1998; up to at least 1 year: Dixon et al., 1999), and fluid percussion injury (up to at least two months: Bramlett et al., 1997; up to at least 1 year: Smith et al., 1997). This long-lasting and progressive character of damage has been found in patients as well (Anderson et al., 1995; Gale et al., 1995; Bigler et al., 1997).

The character of the water maze deficits

Considering the widespread damage and disturbance of brain function, it is expected that other factors apart from spatial navigation disturbances affected performance in the water maze. Damage to the hippocampus (Morris et al., 1990; Moser et al., 1993), the entorhinal cortex (Rasmussen et al., 1989; Eijkenboom et al., 2000a), the parietal cortex (Save et al., 1992) or the fimbria (van der Staay et al., 1989; van Rijzingen et al., 1996) have all been reported to induce spatial learning impairments. In this study, the visual, motor and sensorimotor cortices were also lesioned. In accordance with the sensorimotor cortical damage, grasping reflexes of the hind paw and forelimb flexion

were disturbed immediately after surgery. In a previous study, we found that 35 days after surgery, SDH rats were still impaired in those reflexive sensorimotor behaviours (Eijkenboom et al., 2000b).

These reflexive impairments, however, did not affect swimming during the nonspatial swimming task in the aquarium (this study), i.e. all rats could swim for 5 min in the water tank, or the speed of swimming in the water maze task (this study; see also Eijkenboom et al., 1999, 2000b). Other behavioural changes, such as decreased motivation, increased thigmotaxis, or hyperactivity, might have interfered with our measures of spatial acquisition. The SDH deficit observed with the measure "distance to the platform" suggests that during the tests started at 1, 2, and 4 weeks after surgery the SDH rats swam more along the wall of the tank, i.e. wall-hugging, than the SH rats did. At 8 and 18 weeks post-injury the SDH groups did not differ from the SH groups in the distance to platform. This could indicate that the differences in degree of deficit in distance travelled seen during the first weeks and during the 18th week after surgery might be related to the presence or absence of wall hugging, respectively. Moreover, damage to the visual cortex and the optical tract suggests that SDH rats might have suffered from visual dysfunctions, which might have affected the water maze performance as well.

In addition, it is possible that in a chronic experiment, the time of year influences the way rats behave in the Morris water escape task, even though the conditions in the animal and experimental room were kept constant. However, the rats were not tested chronologically with respect to the length of the surgery-test intervals. Further, ageing-related changes in cognition are not very likely to have interfered with the measurement of recovery after brain injury in this study. Age-related deficits in the allocentric and reference memory version of the Morris water maze task in rats have been observed to occur at the ages of 12–18 months or older (Aitken and Meaney, 1989; Steward et al., 1989; Brandeis et al., 1990).

Validity of the behavioural effects of the SDH model

The differing results at 8 and 18 weeks after surgery are very interesting, as they may bear similarity to the clinical situation: fluctuations in recovery and deterioration of cognitive deficits were seen in some patients after stoke-induced brain damage (Kotila et al., 1986). In this study, the second phase of deterioration might be caused by a phase of progressive brain damage with late onset. In previous rat studies, progressive brain atrophy was observed up to 12 months after unilateral fluid percussion (Smith et al., 1997), and up to 15 months after unilateral entorhinal cortex lesions (Peterson et al., 1994). This explanation would fit the histological result regarding reactive astrocytes, i.e. at 8 weeks GFAP labelling was not found in the hippocampus, entorhinal cortex, optical tract and piriform cortex, whereas at 4 and 18 weeks after surgery these brain regions were strongly labelled for GFAP. However, a quantitative inspection of brain damage is needed for strengthening these notions.

CONCLUSION

Bilateral SDH placed above the somatosensory cortex of rats resulted in spatial navigation impairments that seemed to recover completely at 8 weeks after surgery. At 18 weeks after surgery, however, a second phase of deterioration of performance due to SDH occurred. The rat SDH model provides an interesting tool for investigating spontaneous recovery processes of spatial navigation (8 weeks after injury), but also for progressive brain dysfunction considering the second phase of behavioural impairments seen at 18 weeks after injury.

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